Safety Data Sheet

Diphtheria toxin

Division of Safety
National Institutes
of Health

WARNING!

THIS COMPOUND IS HIGHLY TOXIC. IT IS READILY ABSORBED BY VARIOUS BODY TISSUES THROUGH THE SKIN AND RESPIRATORY TRACT. AVOID FORMATION AND BREATHING OF AEROSOLS.

LABORATORY OPERATIONS SHOULD BE CONDUCTED IN A FUME HOOD, GLOVE BOX, OR VENTILATED CABINET.

AVOID SKIN CONTACT: IF EXPOSED, WASH WITH DILUTE WARM ACETIC ACID OR BORIC ACID SOLUTION. AVOID RUBBING OF SKIN OR INCREASING ITS TEMPERATURE.

FOR EYE EXPOSURE, IRRIGATE IMMEDIATELY WITH WARM ISOTONIC SALINE. FOR INHALATION, REMOVE VICTIM PROMPTLY TO CLEAN AIR. REFER TO PHYSICIAN.

IN CASE OF LABORATORY SPILL, WEAR PROTECTIVE CLOTHING DURING CLEANUP. AVOID SKIN CONTACT OR BREATHING OF AEROSOLS. DISPOSE OF WASTE SOLUTIONS AND MATERIALS APPROPRIATELY.

A. Background

Diphtheria toxin (DT) is a toxic protein produced by the bacillus *Corynebacterium diphtheriae* and is the causative agent in the etiology of diphtheria. This organism is found in the upper respiratory tract of men, cattle, and horses where its proliferation results in the formation of a pseudomembrane. Symptoms are not localized and death results from damage in a variety of internal organs. DT is highly toxic to man, rabbit, guinea pig, cat, dog, pigeon, and young chicken while the rat and mouse are resistant (Gabliks and Solotorovsky, 1962). The main uses of DT are in the commercial preparation of toxoid and antitoxin, and in research on certain phases of intracellular protein synthesis whose inhibition appears to be the major mechanism of toxic action of DT.

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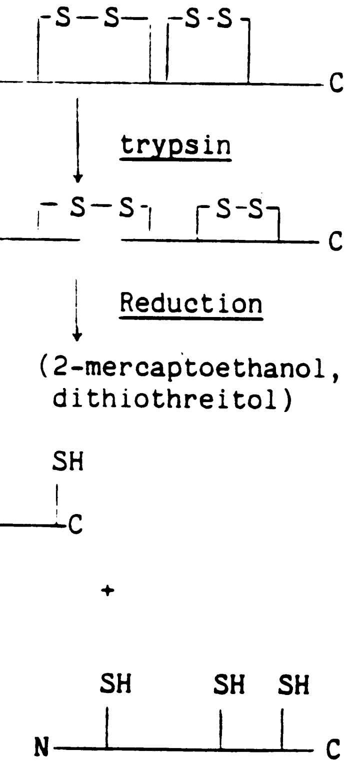
Earlier work on the chemical properties and mechanism of action of DT has been reviewed (Collier, 1975; Pappenheimer, 1977).

B. Chemical and Physical Data

1. Chemical Abstract No.: None.

2. Synonyms: None.

3. Chemical structure and molecular weight: DT is a single-chain protein with molecular weight of 63,000, containing two disulfide linkages. In the intact organism or in cell cultures this protein is toxic but has no enzymic activity[A] in cell-free systems. In the presence of some proteolytic enzymes (e.g., trypsin) and sulfhydryl compounds (both present in the animal and in cell cultures) it undergoes a conversion which is represented as follows (taken from Collier, 1975).



Name	Mol. Wt.	Toxic	Enzymic Activity
Intact toxin	63,000	+	-
Nicked toxin	63,000	+	-
Fragment A	24,000	-	+
Fragment B	39,000	-	-

The N-terminal amino acid of DT (and of Fragment A) is glycine, and the C-terminal amino acid of Fragment A is arginine. The complete amino acid sequence of Fragment A has been determined (DeLange et al., 1976), while only portions of the sequence of Fragment B are known at this time (Falmagne et al., 1979). The "nicking" of intact toxin by trypsin may be a simple peptide

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For explanation of "enzymic activity" see F, below.

bond splitting, or a few amino acids may be released during this process. A dimer of DT, with equal or slightly lower toxicity and dissociated to DT by dithiothreitol, is found in some but not all toxin preparations (Goor, 1968; Gill and Dinius, 1971). For a more detailed description of various sites on Fragment B and their postulated functions, see Proia et al., 1980. For toxicological information on DT and its fragments see Section F.

Density: No data.

Absorption spectroscopy: No data.

Isoelectric point: The most recent data show isoelectric points for DT, Fragment A, and Fragment B of 6.0, 5.25, and 6.8, respectively (Proia et al., 1980).

Volatility: May be considered negligible.

Solubility: Soluble in water or dilute salt solutions.

Description: White powder or crystals. Various crystalline forms (needles, plates, shields) have been described, depending on method of crystallization, but it is uncertain which of these correspond to pure toxin (Pope and Stevens, 1958).

Boiling point, melting point: Not applicable.

Stability: DT is stable upon dialysis at pH 6-8 but toxicity is destroyed if the pH drops to below 5.5 (Norlin, 1955). Fragment A is remarkably stable to temperature and pH (not coagulated at 100°C over the range of pH 3-11, and little loss of toxicity), to proteolytic enzymes, and to denaturing agents such as guanidine and sodium dodecylsulfate (Gill and Dinius, 1971). In contrast, Fragment B is readily denatured with coagulation, but appears to be fairly heat stable (Cukor et al., 1973), and stable on prolonged storage at -20°C in 6 M guanidine hydrochloride. Upon dialysis of the latter solution at 4°C, it remains stable for several hours at room temperature (Burgoyne et al., 1976). It is readily attacked by proteolytic enzymes.

Chemical reactivity: DT produces positive tests with the usual protein reagents (biuret, ninhydrin, etc.) Nitration (with tetranitromethane) of one tyrosine residue in the A fragment of DT results in loss of toxicity and enzymic activity. Reductive methylation (sodium borohydride and formaldehyde) of lysine residues decreases toxicity and enzymic activity. Modification of one (of a total of two) tryptophan residue of the A fragment by 2-hydroxy-5-nitrobenzyl bromide also destroys the enzymic activity (Beugnier and Zanen, 1977; Michel and Dirkx, 1977).

The effect of sulfhydryl compounds (2-mercaptoethanol, dithiothreitol) on the disulfide linkages has already been mentioned (Section B. 3). Treatment with formaldehyde converts DT to the non-toxic toxoid. No chemical reactions which would be useful in decontamination, other than instability to acid, have been mentioned in the literature. DT produces insoluble precipitates with purified antitoxin.

13. Flash point: Not applicable.

14. Autoignition temperature: Not applicable.

15. Explosive limits in air: Not applicable.

Fire, Explosion and Reactivity Hazard Data

1. As a protein, DT is inactivated under conditions of fire. It gains entry into the body through lesions in the epithelium and possibly through inhalation of dusts, though not through the gastrointestinal tract. Therefore, fire-fighting personnel should wear complete protective clothing and air-supplied respirators with face masks.

2. Flammability is likely to be low.

3. Conditions contributing to instability (and detoxification) are high temperature and acid.

4. No hazardous decomposition products are known.

Operational Procedures

It should be emphasized that this data sheet and the NIH Guidelines are intended as starting points for the implementation of good laboratory practices when using this compound. The practices and procedures described in the following sections pertain to the National Institutes of Health and may not be universally applicable to other institutions. Administrators and/or researchers at other institutions should modify the following items as needed to reflect their individual management system and current occupational and environmental regulations.

1. Chemical inactivation/decontamination: No validated method reported. Formaldehyde converts DT to non-toxic toxoid, and DT is unstable in acid solution. DT is inactivated by alkali (e.g., 0.1 N sodium hydroxide).

2. Disposal: It may be possible to decontaminate waste streams containing DT before disposal (see above). No waste streams containing DT shall be disposed of in sinks or general refuse. Surplus DT or chemical waste streams contaminated with DT shall be handled as hazardous chemical waste and disposed of in accordance with the NIH chemical waste disposal system. Nonchemical waste (e.g., animal carcasses and bedding)

containing DT shall be handled and packaged for incineration in accordance with the NIH medical-pathological waste disposal system. Potentially infectious waste (e.g., tissue cultures) containing DT shall be disinfected by heat using a standard autoclave treatment and packaged for incineration, as above. Burnable waste (e.g., absorbent bench top liners) minimally contaminated with DT shall be handled as potentially infectious waste and packaged for incineration, as above. Absorbent materials (e.g., associated with spill cleanup) grossly contaminated shall be handled in accordance with the chemical waste disposal system. Radioactive waste containing DT shall be handled in accordance with the NIH radioactive waste disposal system.

3. Storage: Store solid DT and its solutions in dark-colored, tightly closed containers under refrigeration. Avoid exposure to light and moisture.

Monitoring and Measurement Procedures Including Direct Field Measurements and Sampling for Subsequent Laboratory Analysis

1. Sampling: No data.

2. Analysis: One of the most sensitive assays of DT is the Schick Test, used clinically in the establishment of presence or absence of immunity to diphtheria. It is based on the production of erythema at the site of intracutaneous inoculation of rabbits or guinea pigs with DT. The minimal reactive dose of U.S. Standard Diphtherial Toxin is 2.5×10^{-5} flocculating units, corresponding to about 50 pg of DT. A reversed passive hemagglutination assay, using formalinized sheep erythrocytes sensitized with purified antitoxin, has been described (Holmes and Perlow, 1975). This assay can detect less than 20 pg of DT. It is feasible that a test based on the enzymatic activity of DT could be devised. Such a test should be highly sensitive and specific.

Biological Effects (Animal and Human)

1. Absorption: DT is absorbed in clinical cases of diphtheria through lesions in the epithelium at sites of infection, and experimentally by parenteral injection and probably through the respiratory tract if inhaled as dust. It is ineffective when ingested because of its instability at the acid pH of the stomach.

2. Distribution: DT labelled with ^{125}I and injected intravenously into guinea pigs is distributed uniformly in all tissues within 2-12 hours after administration except for little or no accumulation in the central nervous system and higher than expected concentrations in the kidney (Masouredis, 1959; Baseman et al., 1970).

3. Metabolism and excretion: The mechanism of metabolism of DT ha not been investigated but presumably consists of proteolysis. Loss of plasma radioactivity from intravenously administered DT in guinea pigs is biphasic, the first phase amounting to 46% pe hour (half time 1.5 hrs) and this amounts to 80% of plasma activity; the remainder is lost at a rate of 3.5-2.3% per hour (half time 20-30 hrs). The rate of disappearance from tissues parallels that from the circulation. Urinary excretion of the radiolabel is at a rate of 3.63% per hour and is in the form of nontoxic and not protein-bound material (Masouredis, 1959).

4. Toxic effects: The toxicity of DT in the guinea pig and rabbit, by intravenous or subcutaneous injection, is of the order of 0. µg/kg.[A] Other species susceptible to DT are cat, dog, man, pigeons, and young chickens, while the rat and mouse are resistant. (Doses about 1,000 times higher are required in these species to produce comparable toxicity.) The same differences obtain in the susceptibilities of primary kidney cell cultures from these species (Gabliks and Solotorovsky, 1962). Collier (1975) has stated "...it may be calculated that a few micrograms is sufficient to cause death of an unimmunized adult human." As mentioned above, DT is ineffective when administered orally because of its instability at acid pH.

   There is a marked lag period between administration of DT and the appearance of toxic symptoms. Even on administration of several thousand times the lethal dose this lag period may last for 4-5 hours, and there are no animal deaths prior to 10 hours. However, the toxin appears to be absorbed much more rapidly into cells, since administration of antitoxin one hour after DT does not prevent death. Following the latent period one finds profound morphological changes in many organs such as heart, adrenal, kidney, liver, pancreas, diaphragm, and nervous tissue. It is generally believed that there is no particular target organ. Gross symptoms following the latent period are gradual muscular weakness and lethargy; these become progressively more pronounced until the animal goes into shock and dies. Experiments with human HeLa cells in tissue culture, using ^{125}I-labeled DT, indicate that there is an initial rapid reaction between a "recognition site" on Fragment B and specific plasma membrane receptors on the cell, followed by a slow irreversible process during which DT is nicked, fragmented, and Fragment A enters the cytosol (Boquet and Pappenheimer, 1976).

   The mechanism of toxic action of DT consists of a potent inhibition of protein synthesis such as is found also with plant lectins (abrin, ricin). In contrast with these latter toxins

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[A]The very few toxicity data in the literature (Baseman et al., 1970; Pappenheimer and Gill, 1972, 1973; Collier, 1975) are in terms of minimal lethal dose rather than LD50. It is estimated that MLD and LD50 are not too different. The only reported LD50 is 0.015-0.064 µg protein nitrogen per 250 g guinea pig (Masouredis, 1959) but there is no published figure for the percentage of protein nitrogen in DT.

the mechanism is quite well understood, and is enzymic in nature: DT catalyzes the reaction of "elongation factor 2" (EF2) with nicotinamide adenine dinucleotide (NAD, diphosphopyridine nucleotide, coenzyme 1) in the following reaction:

$$EF2 + NAD \rightleftharpoons ADP\text{-}Rib\text{-}EF2 + nicotinamide + H^+$$

The equilibrium position of this reaction is far to the right, and the resulting complex is inactive in promoting the translocation event on ribosomes. DT thus acts as an enzyme or, more strictly speaking, as a proenzyme since intact DT needs to be "nicked" and fragmented for the A fragment to exert its inhibitory role on protein synthesis (see scheme in B. 3). The process is discussed in detail by Collier (1975) and Chung and Collier (1977). As may be expected from the above, intact toxi or nicked toxin is required to produce toxic effects in the animal or in whole cell cultures, while the A fragment alone is sufficient for inhibition of protein synthesis in cell-free preparations. Mouse and rat cells seem to contain little or no EF2 and this fact may explain the lack of susceptibility of these species to DT.

5. Carcinogenic effects: No carcinogenic effects of DT have been reported. The possibility that DT may exhibit selective sensitivity to malignant cells (such as has been found with abrin an ricin) has been raised, but evidence on this subject has been largely negative (Pappenheimer and Ranall, 1975; Venter and Kaplan, 1976).

6. Mutagenic and teratogenic effects: None have been reported.

Emergency Treatment

1. Skin and eye exposure: For skin exposure, remove contaminated clothing and wash skin with dilute acetic acid or boric acid solution. Avoid rubbing of skin or increasing its temperature. For eye exposure, irrigate immediately with copious quantities of running water for at least 15 minutes. Obtain ophthalmological evaluation.

2. Ingestion: Drink plenty of water or milk. Refer for gastric lavage.

3. Inhalation: Remove victim promptly to clean air. Administer rescue breathing if necessary.

4. Refer to physician at once. Consider treatment for pulmonary irritation, and prompt administration of diphtheria antitoxin.

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